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Sempervirens, and Gelsemium Poisoning.

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IS GELSEMIC ACID IDENTICAL WITH AESCLULIN?

With Observations on the Preparation, Properties and Recovery, when Absorbed, of the Important Constituents of Gelsemium Sempervirens, and Gelsemium Poisoning.

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In a former number of this journal (Jan., 1870) the writer announced that *Gelsemium Sempervirens* contained a non-nitrogenized principle, of an acid reaction, which was named *gelseminic*, or *gelsemic acid*; and also a strongly basic principle, which was named *gelsemia*, or *gelsemine*.

From a subsequent examination of the constituents of the plant, M. Sonnenschein and Charles Robbins concluded that the so-called *gelsemic acid* was identical in properties and composition with the glucoside *aesculin*, found in the bark of the horse-chestnut, and certain other barks ("Ber. der Deut. Ges.", Sept. 1876, 1182).

Before examining this question of claimed identity, the method, of several employed, which we have more recently found the most satisfactory for the extraction of the two important constituents of the plant, will briefly be given.

Preparation.—100 grams of the powdered dry root are macerated for two days in 400 cc. of a mixture of equal parts of water and alcohol of .815, the mixture being slightly acidulated with acetic acid and occasionally warmed and agitated. The liquid is strained through muslin, and the solids well washed with water containing a little alcohol, the washings being collected with the first liquid. The whole is evaporated to about 200 cc., and allowed to stand until the resinous matter has deposited. The liquid is then filtered, concentrated to about 80 cc. and, if necessary, again filtered.

1. *The non-nitrogenized principle.*—This is extracted from the concentrated liquid, while it still has an acid reaction, by ether, in the

usual manner, using three or four volumes of the liquid in two or three portions.

The crystals obtained on evaporation of the ether are washed with a little absolute alcohol, which readily dissolves the adhering coloring matter. The residue may be further purified by a second extraction by ether.

A very good method of purifying the substance, especially from the last traces of the alkaloid, is to dissolve it in about two hundred and fifty parts of water, by the aid of a few drops of ammonia, and then treat the clear solution with diluted hydrochloric acid, added drop by drop until the point of neutralization is almost, but not fully reached, taking care that the first cloudiness or precipitate produced has fully crystallized before adding another drop of the acid. The crystals are collected on a small filter and washed with a little cold water.

2. *Gelsemine*.—The liquid from which the former principle was extracted is gently warmed until the dissolved ether has been expelled. It is then rendered slightly alkaline by sodium hydrate or carbonate, and the liberated base extracted by ether, which is allowed to evaporate spontaneously.

The impure alkaloid thus obtained is dissolved, by the aid of a few drops of hydrochloric acid, in about 12 cc. water, the solution filtered, and the filtrate treated with a very slight excess of sodium hydrate, when a large portion of the alkaloid will separate as a pure white precipitate. This is quickly collected on a filter and washed with pure water. The remaining portion of the alkaloid is extracted from the filtrate by ether or chloroform.

From the dried root of the plant we obtained, after this manner, 25 per cent. of gelsemine and 50 per cent. of the acid principle.

Is Gelsemic Acid Identical with Aesculin?

For the purpose of answering this question various samples of the gelsemium principle were examined comparatively with a sample of *aesculin* prepared by E. Merck, of Darmstadt. It consisted of a spongy, sparkling white mass of minute (microscopic) needles and prisms. On comparing it with a somewhat colored preparation obtained from horse-chestnut bark, the substances presented essentially the same properties in the several respects examined.

This comparative examination showed :

- A. These principles agree more or less in the following respects :

1. Both substances are readily soluble in the *caustic alkalies*, forming solutions which have a yellow color by transmitted, and appear blue by reflected light, the fluorescence appearing in both instances even in very highly dilute solutions, being distinctly marked in a 100,000th solution.

The fluorescence of the gelsemium principle, however, is *greenish blue*, whilst that of *aesculin* is deep *sky blue*. The fluorescence in both instances is destroyed by free acids

2. *Nitric acid* dissolves both substances with a yellow color, and the solutions, when treated with excess of ammonia, assume a deep red color. This red coloration may be obtained from even the 1-50,000th of a grain of either substance.

The nitric acid solution of the gelsemium principle, when present in sufficient quantity, has an orange-red color, whereas that of *aesculin* is yellow.

B. They differ more or less in the following properties:

1. *Crystallization.* — The gelsemium compound very readily assumes the crystalline form, even in the presence of comparatively large proportions of resinous matter, whereas *aesculin* crystallizes with some difficulty, even from pure solutions. Thus, the 1-10,000th grain of the former substance, when separated from one grain of solution, is left in the form of needles, whilst crystals can only be obtained from rather strong solutions of *aesculin*, and then usually appear as transparent spherical masses, with some dense tufts of short prisms.

2. *Solubility.* — *a. Water.* The gelsemium substance, when pure, requires 2,912 parts of water for solution, even when excess of the powder is kept in contact with the liquid at a temperature ranging from 18° to 24°C. (65° to 75°F.), for twenty-four hours.

Under like conditions, Merck's *aesculin* dissolved in 401 parts of water. According to Trommsdorff *aesculin* requires 576 parts of water for solution, but according to Minor it dissolves in 300 parts (Gmelin's Hand-Book, xvi, 22).

b. Ether. One part of the gelsemium principle was readily taken up by 330 parts of *ether* of sp. gr. .728, whereas *aesculin* required at least 36,000 parts of the same fluid for solution.

So also, the former principle is rather freely soluble in *chloroform*, whilst the latter is nearly or wholly insoluble in this liquid.

3. *Sulphuric acid* dissolves the gelsemium substance to a more or less yellowish solution, which when warmed in a water oven (if the

substance is pure) undergoes little or no change. \AA esculin readily dissolves in the acid, but on warming the solution it quickly acquires a brownish or chocolate color and becomes charred.

If a drop of aqueous ammonia be allowed to flow into a drop of the sulphuric acid solution of the gelsemium compound, a dirty white deposit or cloud of very minute crystalline needles separates at the margin of contact of the liquids.

If only a minute drop of the acid be employed and excess of ammonia be avoided, even the 1-10,000th grain of the substance will in this manner yield a very satisfactory deposit of needles. If the drop of liquid be allowed to evaporate, these crystals may be re-examined, even several times, by moistening the residue with a minute drop of water, which will quickly dissolve the ammonium salt, whilst the needles will remain, they being apparently insoluble under these conditions.

This reaction is highly characteristic of the gelsemium principle.

A sulphuric acid solution of *æsculin* under like conditions fails to yield any crystals, unless a comparatively large quantity is present and the mixture be evaporated to about dryness, when transparent nodular masses with some groups of prisms may appear.

4. *Hydrochloric acid* fails to dissolve or act upon the gelsemium compound, even under the heat of a water bath. \AA esculin is readily soluble in this acid.

In the following liquid reactions the results refer to the behavior of a few drops of 1-100th solutions of both principles.

5. *Nitrate of silver* produces in a solution of the gelsemium principle a copious brownish-yellow precipitate, which soon darkens in color, and finally the mixture becomes deep blue-black, due to the reduction of the silver salt. This reaction will manifest itself, after a time, even in a 1-50,000th solution.

\AA esculin yields from a 1-100th solution, a slight dirty yellow precipitate, which remains unchanged for several minutes; it then slowly darkens.

6. *Corrosive sublimate* throws down a copious yellowish precipitate, from which the organic acid quickly separates as large tufts of needles.

\AA esculin fails to yield a precipitate or crystals.

7. *Bromine in bromohydric acid* produces a copious green deposit, which quickly acquires a blueish and finally a brownish color.

\AA esculin yields a slight yellow precipitate, which becomes yellowish-gray.

8. *Sulphate of copper* causes in a solution of the acid a dirty brown precipitate, which soon assumes a dull red color, and crystalline needles separate.

Aesculin yields a blueish white deposit, which undergoes little or no change.

9. *Acetate of lead* (neutral) produces a copious yellow precipitate, which after a time is partly changed into very minute star-like groups of crystals. These are insoluble in ammonia, but readily soluble in acetic acid, being quickly replaced by slender needles of the free acid.

Aesculin yields a somewhat similar precipitate, but no crystals were obtained.

10. *Physiological action.*—*a.* 0·010 gram (about $\frac{1}{6}$ grain) of the gelsemium principle was administered hypodermically to a frog. After a few minutes the eyes were fluorescent, and the animal seemed sluggish; after half an hour it was somewhat excited and apparently weak. No other marked symptoms were noticed during the several hours the animal was observed.

A similar quantity of *aesculin* produced fluorescence of the eyes, but no other apparent effect.

b. 0·033 gram ($\frac{1}{2}$ grain) of the gelsemium substance, prepared by precipitation with hydrochloric acid, being injected into the peritoneum of a frog, was quickly followed by violent agitation; the animal became rigid, and reflex action was markedly diminished. In five minutes the animal was apparently lifeless; there was rigidity of the abdominal muscles, which slowly relaxed. After forty minutes, the heart had ceased to beat, was relaxed and not irritable.

c. A similar quantity, injected into the posterior lymph sack, was quickly followed by great agitation and general prostration. In fifteen minutes active voluntary movements appeared, and there was marked fluorescence of the entire eye-ball. After forty-five minutes the heart was found still beating, but ceased fifteen minutes later.

In another experiment, a like quantity caused a complete cataleptic condition and death within ten minutes.

d. 0·033 gram of *aesculin* injected into the peritoneum of a similar frog produced *no apparent effect*, other than a marked fluorescence of the eyes, which continued some fifteen hours.

For these physiological experiments, I am much indebted to Dr. Edward T. Reichert. These results, in regard to the gelsemium principle, confirm in a measure those previously obtained by Dr. Isaac Ott. —(*American Practitioner*, 1877.)

Conclusions.—It is needless to add that the only conclusion from the foregoing comparative results is that the principles examined are very different substances. Hence the name *gelsemic acid* will be retained provisionally for the gelsemium principle.

Gelsemine.—As supplementary to our former paper on the subject, some of the more important properties and reactions of gelsemine may be mentioned.

In its pure state gelsemine is a colorless, odorless solid, having a persistent bitter taste. It has not yet been obtained in the crystalline state. At something below 100°C. it fuses to a colorless liquid. Gelsemine completely neutralizes acids, forming salts, most of which are freely soluble in water and alcohol.

The pure alkaloid is soluble, under ordinary conditions, in 644 parts of water. It is freely soluble in ether and in chloroform.

The most characteristic reactions of the solid alkaloid are the following:

1. *Sulphuric acid* dissolves gelsemine with a reddish or brownish color to a solution which after a time assumes a pinkish hue. If the solution be warmed on a water-bath, it acquires a more or less purple or chocolate color.

If a small crystal of *potassium bichromate* be slowly stirred in the sulphuric acid solution, reddish-purple streaks are produced along the path of the crystal. If the potassium salt be used in the form of *powder*, or, as advised by Sonnenschein and Robbins, be replaced by *ceric oxide* (CeO_2 formerly Ce_3O_4), the purplish or reddish-purple coloration manifests itself more promptly and strongly, and may be obtained from even the one ten-thousandth grain or less of the pure alkaloid. For the detection of these minute quantities, however, it is essential that only very minute quantities of the acid and powder be employed.

This reaction of gelsemine—as remarked by Sonnenschein and Robbins, who first observed it with the cerium compound—resembles somewhat that of strychnine; but these alkaloids could not thus be confounded.

2. *Nitric acid* causes gelsemine to assume a brownish-green, quickly changing to a deep green, color, which slowly diffuses itself through the liquid. Almost the least visible quantity of the alkaloid, if touched with only a very minute drop of the acid, will yield this green coloration in a marked degree.

This reaction readily distinguishes gelsemine from strychnine and the other alkaloids.

Solutions of the *salts* of gelsemine are colorless, and have the strongly bitter taste of the alkaloid. These solutions yield precipitates with a number of different liquid reagents, even in some instances when highly dilute; but in no instance is the reaction peculiar to this alkaloid.

Physiological action.—0.008 gram ($\frac{1}{8}$ grain) of gelsemine, administered hypodermically to a cat, caused very marked symptoms in fifteen minutes, and death in one hour and a half.

0.010 gram, given to a frog, produced, after half an hour, great prostration, followed by tetanic convulsions and death in about four hours.

0.033 gram of the alkaloid, in the form of chloride, was injected into the peritoneum of a frog. The animal soon opened its mouth convulsively, the jaws fell at intervals, and there was quickly great muscular prostration. In twenty minutes the body was completely relaxed; the muscles not irritable under pricking; reflex action was greatly diminished, and life seemed to be extinct. On opening the thoracic cavity it was found that the heart had been arrested in diastole and was not irritable.

Gelsemium Poisoning.—The cases of gelsemium poisoning thus far reported have been, with two or three exceptions, the result of accident or ignorance. The preparation of the drug most frequently employed is the *fluid extract*, each fluidounce of which represents 480 grains of the dried root. This preparation, as found in the shops, as we have heretofore shown (this journal, 1877, 151), usually contains .2 per cent. of gelsemine and .4 per cent. of gelsemic acid.

Of *twenty-five* cases of gelsemium poisoning that we have collected, (some private), *thirteen* proved fatal. The *fatal period* varied from *one hour* to about *eight hours*. Very small quantities of the drug may cause death. A case is reported in which a quantity of a tincture equivalent to about *twelve minims* of the fluid extract proved fatal to a child aged three years. In another instance, four doses of *fifteen minims* each of the fluid extract, repeated at short intervals, caused the death of a healthy man in less than four hours after the last dose was taken. So, also, a teaspoonful of the same preparation proved fatal to a woman.

There is no chemical *antidote* known for this kind of poisoning.

The application of *electricity* has in several instances proved very beneficial. In others, the use of *morphine* hypodermically has been attended with good results.

Chemical Analysis.—In gelsemium poisoning the gelsemic acid and the alkaloid are both absorbed, and enter the blood apparently in the proportion in which they are found in the plant. Hence, in poisoning by the drug, it becomes necessary to direct the examination for the absorbed poison to the recovery of both these principles. This is the more important, since the acid is apparently not much less poisonous than the alkaloid, and so readily reveals its presence by its fluorescent properties.

The general method for the recovery of strychnine and like substances from the blood and tissues is about equally applicable for the recovery of the gelsemium principles. As these substances are readily soluble both in ether and chloroform, either of these liquids may be employed for the extraction. The gelsemic acid would, of course, be found in the ether extract from the prepared solution, while it still had an acid reaction; whilst the alkaloid would be extracted from the solution after it had been rendered alkaline.

In applying the tests for gelsemic acid to the ether residue from the acid solution it should be remembered that although the reaction of the nitric acid and ammonia test is common to gelsemic acid and æsculin, yet when obtained from an *ether* extract, it is characteristic of the former substance, since æsculin is not extracted by ether.

The blood and liver of a cat which had been killed, after several hours, by the drug, were examined, in the main, after this general method. The first ether extract, in both instances, was distinctly fluorescent, and on evaporation left the gelsemic acid, in part at least, in its crystalline state. The true nature of these crystals was readily established by the appropriate tests. So, also, about equally satisfactory evidence of the presence of gelsemine was obtained from the ether residues from the alkaline solutions.

As a conclusion from these and other similar results, it would appear that in gelsemium poisoning evidence of the presence of the poison in the blood may be more readily and fully obtained than in the case of any of the other vegetable poisons.

Philadelphia, June, 1882.

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